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MESSAGE

Please find the enclosed Miscellaneous Letter regarding U.S. Patent Application No. 09/993,326.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Mark M. Wang et al.

Serial No.: 09/993,326

Filed: November 14, 2001

For: METHOD OF SEPARATING PARTICLES USING AN OPTICAL GRADIENT (As Amended)

Group Art Unit: 1651

Examiner: Jon P. Weber

Allowed: November 10, 2003

Issue Fee Due: February 10, 2004

MISCELLANEOUS LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In reviewing the Form PTO/SB/08A accompanying the November 10, 2003 Notice of Allowance, it appears that the following reference: *Imasaka et al*, "Optical Chromatography," Analytical Chemistry, Vol. 67, No. 11, pp. 1763-65, June 1, 1995 was missing from the May 23, 2002 Information Disclosure Statement. A copy of the *Imasaka et al.* reference is included herewith. Applicants request that that above-identified reference be considered by the Examiner such that the reference may appear on the face

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Patent
Attorney Docket: 302,670-6
(prev 265/071)

of the patent issuing from this Application.

If there are any questions regarding the submission of this paper, please call the undersigned at (949) 737-2926.

Respectfully submitted,

O'MELVENY & MYERS LLP

Dated: 11/26/03

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IR1:1050135.1

Accelerated Articles

Anal. Chem. **1995**, *67*, 1763–1765

Optical Chromatography

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A new and potentially useful method for separation of particles by optical radiation pressure is described and demonstrated in this study. A laser beam is focused into the solution, which contains particles counterflowing coaxially in a capillary. The particle is focused into the center line of the laser beam by radiation pressure. The particle is turned around, accelerated, passed through a beam waist, decelerated by a liquid flow, and drifts, at which point the radiation pressure is identical to the force induced by the liquid flow, resulting in separation of particles as a function of size.

In 1906 Tswett reported on a separation technique that is now generally called chromatography.[1] Classical adsorption chromatography involves the adsorption of a substance (for example, a dye) to the solid support or solid phase. The adsorbed compound can then be eluted (desorbed) from the solid support by passing a liquid solvent or mixture of solvents over the solid phase which desorbs the compound from the support, thereby affecting a separation and purification. Chromatography has been studied by many researchers in the interim years and is now used almost universally in chemical analysis and separation science. Chromatography, when used in the classical way, however, has serious limitations that have not yet been (and may never be) overcome. Some of the limitations of chromatography are as follows: (1) Columns must be replaced frequently in order to optimize separation, making the technique time consuming. (2) Retention times must be measured for a series of compounds prior to the analysis of an unknown sample, since it cannot be precisely calculated from its physical properties. (3) Resolution is limited by diffusion in the column and cannot be greatly improved by extending the separation time. (4) Biological cells and large molecules that are of biochemical interest are poorly separated by chromatography as well as by other techniques such as field-flow fractionation or flow cytometry. (5) Detection efficiencies are well below unity. (6) The concentration detection limit is rather poor, even in state-of-the-art chromatographic techniques, such as capillary electrophoresis combined with laser fluorometric detection, thus making preconcentration a necessity, requiring additional instrument and time. (7) It is desirable to recover the sample at the same concentration that was used in the starting run, so that further experiments can be performed with the sample. (8) The sample is obtained in an eluent, usually in fractions, and is in a diluted state. (9) It is usually difficult or

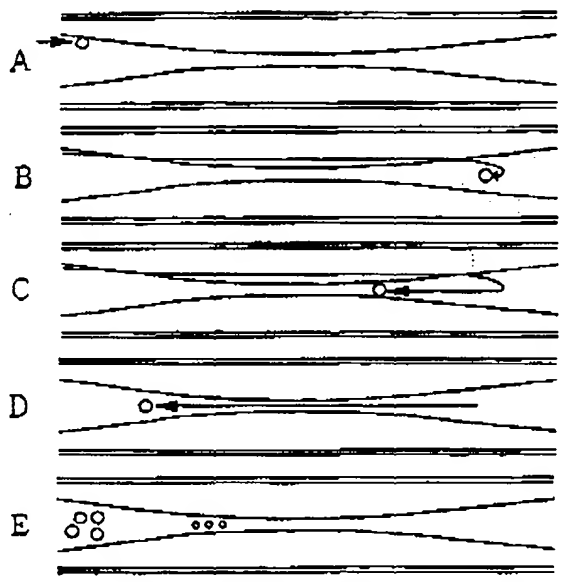

Figure 1. Schematic motion of particle. The laser beam is introduced from the right-hand side and the liquid from the left-hand side: (A) particle introduction; (B) focus of particle into beam center by gradient force; (C) acceleration of particle; (D) deceleration of particle; (E) particles drifting at equilibrium position.

(1) Pecsok, R. L.; Shields, L. D.; Cairns, T.; McWilliam, I. G. *Modern Methods of Chemical Analysis, 2nd ed.*; John Wiley & Sons: New York, 1968.

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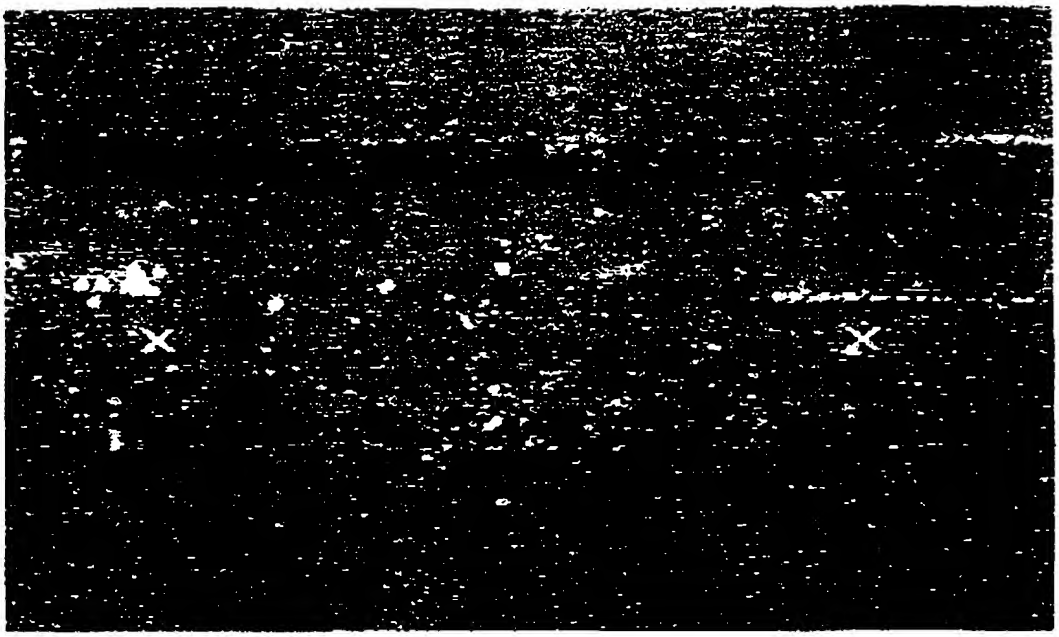

Figure 2. Photograph of particles separated by radiation pressure. The 1-μm particles are localized in a line at around the mark (×) in the right-hand side, and the 3-μm particles at around the mark (×) in the left-hand side. This experiment used a multiline argon laser (454.5–514.5 nm) at 2 W and a 200-μm-i.d. capillary at a 10 μm/s flow rate. Refractive index: polymer beads, 1.59; water, 1.33.

impossible to perform in situ reactions on the compound as it exists in the chromatographic column. (10) Column dimensions, optimally, should be minimized when used as a preseparator in conjunction with a chemical sensor, but the columns are far too long for this to be achieved.

In 1970, Ashkin reported an optical trapping technique.[2] By tightly focusing a laser beam, a particle with a higher refractive index than the medium is trapped at the focal point with radiation pressure. This technique is useful in the sense that a particle can be "held" in place and then subjected to chemical analysis in the micrometer region, e.g., fluorescence measurement of dyes that are adsorbed on a particle.[3] This technique allows the manipulation of a single particle by complete holding like "adsorption", though it is possible to control many particles sequentially by scanning the laser beam.[4]

In this study, we demonstrate a new approach for the separation of particles or molecules using radiation pressure by incomplete holding under a liquid flow. The overall technique affects a chromatographic-like separation but involves principles that are different from classical chromatography. The approach is quite simple: a laser beam is focused into the solution, which contains substances counterflowing coaxially in a capillary. The behavior of the substances is recorded by a video camera equipped with a microscope objective.

RESULTS AND DISCUSSION

The motion of the substance, a particle in this study, under radiation pressure is schematically shown in Figure 1: (A) A particle is introduced into a capillary by a liquid flow. (B) The particle is focused into the center line of the laser beam by the gradient force.[5] (C) The particle is turned around and accelerated by the scattering force.[5] (D) The particle is decelerated by a liquid flow as the particle moves away from the beam waist. (E) The particle drifts when the radiation pressure becomes identical to the force induced by the liquid flow. A photograph of the system at equilibrium is shown in Figure 2. The 1- and 3-μm particles are clearly separated from each other; the 6-μm particles injected are also separated but are located beyond the frame of this picture and are not shown. These data clearly demonstrate that particles can be separated using this technique as a function of particle size.

The present approach has unique characteristics: (1) The particle separation can be easily controlled by altering the beam focusing condition, which corresponds to a replacement of the column in classical chromatography. (2) The position of the particle can be calculated provided its physical properties, e.g., size and refractive index, are known. (3) The separation resolution can be improved by measuring the particle position more accurately by extending the time for measurement. (4) Particles (or large molecules) are more easily separated, although smaller molecules can also be separated by using a stronger laser that is emitting at shorter wavelengths. (5) The separated particle is recorded at all times by a video camera and is detected with an efficiency of 1.0. (6) Separation and concentration are performed simultaneously with no additional instrument and time required. (7) The concentration of the sample recovered by interrupting the laser beam is identical to the original solution. (8) The particles are collected in order of size by decreasing the laser power, and the concentration can be increased. (9) A particle separated in the capillary can be trapped by introducing a second, perpendicular laser beam, thus allowing in situ chemical reactions

(2) Ashkin, A. *Phys. Rev. Lett.* **1970**, *24*, 156–159.
(3) Misawa, H.; Koshioka, M.; Sasaki, K.; Kitamura, N.; Masuhara, H. *Chem. Lett.* **1990**, 1479–1482.
(4) Sasaki, K.; Koshioka, M.; Misawa, H.; Kitamura, N.; Masuhara, H. *Opt. Lett.* **1991**, *16*, 1463–1465.
(5) Ashkin, A. *Biophys. J.* **1992**, *61*, 569–582.

to be performed in succeeding stages (e.g., the detection ...

be reduced to micrometers. This technique addresses most of the problems and limitations of classical chromatography that are described above.

There are several advanced technologies for particle separation, such as field-flow fractionation invented by Giddings.[6] However, optical chromatography as demonstrated herein has several advantages over other separation methods: for example, the driving force, i.e., radiation pressure and liquid flow, can be changed immediately and independently from outside for modification of separation conditions. Moreover, concentration and separation can be performed simultaneously, and then the present method can be applied to diluted samples. The collection efficiency can, in theory, be improved to 100% by increasing the laser output power and by matching the laser beam diameter at the inlet port to the capillary inner diameter, though the efficiency is presently much less than 1%. The separation resolution, which is affected by the beam focusing conditions and the stabilities of the laser and liquid-flow parameters, can be improved by optimizing conditions, though unavoidable limiting factors, e.g., based on a Brownian motion of the molecule in the liquid, exist. It is noted that an irregular or opaque particle can be separated similarly but a light-absorbing, i.e., black, particle is beyond the application of this technique.

The present method may be used in a variety of fields. A straightforward application might be separation of polystyrene RNA. For example, antibody-bound polymer beads are coagulated in the presence of antigen, so that a specific protein can be detected.[7] Since the present method allows the detection of particles at extremely low levels, in theory, a single protein molecule could be detected.

The aim of the present study is not only the demonstration of a new separation technique but also the proposal of a new "field" for chemical reactions and analysis in the micrometer region. For example, ion cyclotron resonance (ICR) mass spectrometry (MS) first isolates a specific ion, which is then dissociated, e.g., by electron impact, to generate daughter ions that are further analyzed by MS. In an analogous manner, a specific molecule could be isolated by optical chromatography and then be optically trapped by a second laser. The molecule can be further reacted, e.g., with an enzyme introduced by an electroosmotic flow, and the products may be further separated and analyzed by optical chromatography. Thus, the present method provides for an ultrasensitive means for structural analysis of large molecules or even particles that cannot be applied to a vapor-phase experiment such as ICR-MS.

Received for review January 17, 1995. Accepted March 19, 1995.[a]

AC950052K

(6) Giddings, J. C. *Anal. Chem.* **1981**, *53*, 1170A–1178A.
(7) Rosenzweig, Z.; Yeung, E. S. *Anal. Chem.* **1994**, *66*, 1771–1776.

[a] Abstract published in *Advance ACS Abstracts*, April 15, 1995.